33333

Access DB# _____

SEARCH REQUEST FORM

Scientific and Technical Information Center

Requester's Full Name: Gailene R. Gabel Examiner #: 76197 Date: 1/17/01
Art Unit: 1641 Phone Number 305-1807 Serial Number: 09/544,776
Mail Box and Bldg/Room Location: 7B15 Results Format Preferred (circle): PAPER DISK E-MAIL

7E12

If more than one search is submitted, please prioritize searches in order of need.

****

Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: Methods and Compositions Using Protein Binding

Inventors (please provide full names): Craig, Roger; Edgar, John (Partners)

Earliest Priority Filing Date: 2/26/99

***For Sequence Searches Only* Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.**

Please search: protein / peptide / antibody / portion / binding interaction / = dependent / protein conformation structure

Please search claims 1, 2, 4, 5, 7, 10, 11
highlighted terms 14, 16-18

See Abstract

Point of Contact:
Mary Hale
Technical Info. Specialist
CM1 12D16 Tel: 308-4258

Thanks Gail

****

STAFF USE ONLY

Searcher: _____
Searcher Phone #: _____
Searcher Location: _____
Date Searcher Picked Up: _____
Date Completed: 2/16
Searcher Prep & Review Time: 10
Clerical Prep Time: _____
Online Time: 17

Type of Search

NA Sequence (#) _____
AA Sequence (#) _____
Structure (#) _____
Bibliographic 1
Litigation _____
Fulltext _____
Patent Family _____
Other _____

Vendors and cost where applicable

STN $294.97
Dialog _____
Questel/Orbit _____
Dr.Link _____
Lexis/Nexis _____
Sequence Systems _____
WWW/Internet _____
Other (specify) _____

PTO-1590 (1-2000)

M. Hakel
511776

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=> fil reg

COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.15       0.15

FILE 'REGISTRY' ENTERED AT 10:24:47 ON 06 FEB 2001
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STRUCTURE FILE UPDATES:    5 FEB 2001  HIGHEST RN 320336-79-6
DICTIONARY FILE UPDATES:   5 FEB 2001  HIGHEST RN 320336-79-6

TSCA INFORMATION NOW CURRENT THROUGH July 8, 2000

  Please note that search-term pricing does apply when
  conducting SmartSELECT searches.

Structure search limits have been increased.  See HELP SLIMIT
for details.

=> fil medl,caplus,biosis,embase,wpids,jicst

COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.31       0.46

FILE 'MEDLINE' ENTERED AT 10:25:18 ON 06 FEB 2001

FILE 'CAPLUS' ENTERED AT 10:25:18 ON 06 FEB 2001
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FILE 'EMBASE' ENTERED AT 10:25:18 ON 06 FEB 2001
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FILE 'WPIDS' ENTERED AT 10:25:18 ON 06 FEB 2001
COPYRIGHT (C) 2001 DERWENT INFORMATION LTD

FILE 'JICST-EPLUS' ENTERED AT 10:25:18 ON 06 FEB 2001
COPYRIGHT (C) 2001 Japan Science and Technology Corporation (JST)

=> s (conformat? or configurat? or arrangement) and (protein or polypeptide
or peptide or amino acid or antibod?)

L1      119925 FILE MEDLINE
L2      119805 FILE CAPLUS
L3       57214 FILE BIOSIS
L4       55055 FILE EMBASE
L5        2260 FILE WPIDS
L6       24224 FILE JICST-EPLUS
```

TOTAL FOR ALL FILES
L7 378483 (CONFORMAT? OR CONFIGURAT? OR ARRANGEMENT) AND (PROTEIN OR POLYP
EPTIDE OR PEPTIDE OR AMINO ACID OR ANTIBOD?)

=> s l7 and (bond? or hold(2a)together or reaction or interact? or activit?)

L8 63988 FILE MEDLINE
L9 68032 FILE CAPLUS
L10 30949 FILE BIOSIS
L11 34908 FILE EMBASE
L12 1285 FILE WPIDS
L13 10534 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L14 209696 L7 AND (BOND? OR HOLD(2A) TOGETHER OR REACTION OR INTERACT? OR ACTIVIT?)

=> s l14 and (post translat? or change? or transfer?) and enzyme?

L15 6592 FILE MEDLINE
L16 6600 FILE CAPLUS
L17 3429 FILE BIOSIS
L18 4909 FILE EMBASE
L19 49 FILE WPIDS
L20 1487 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L21 23066 L14 AND (POST TRANSLAT? OR CHANGE? OR TRANSFER?) AND ENZYME?

=> s l21 and (capture ligand or solid phase substrate)

L22 0 FILE MEDLINE
L23 0 FILE CAPLUS
L24 0 FILE BIOSIS
L25 0 FILE EMBASE
L26 1 FILE WPIDS
L27 0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L28 1 L21 AND (CAPTURE LIGAND OR SOLID PHASE SUBSTRATE)

=> d;s l21 and (label? or luminescen? or fluorescen? or domain or radiolabel? or heavy metal or radioopaque)

L28 ANSWER 1 OF 1 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 2000-565475 [52] WPIDS
CR 2000-558404 [48]; 2000-565459 [48]; 2000-572100 [48]; 2000-572119 [48]; 2000-579184 [48]
DNN N2000-417632 DNC C2000-168512
TI Determining the **conformational** state of a **protein**, comprises contacting the **protein** with a labeled binding **protein** and assessing the labeling of the **protein**.
DC B04 D16 S03

```
IN   COLYER, J; CRAIG, R K
PA   (FLUO-N) FLUORESCIENCE LTD
CYC  89
PI   WO 2000050901 A1 20000831 (200052)* EN   56p    G01N033-68
        RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL
            OA PT SD SE SL SZ TZ UG ZW
         W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES
            FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS
            LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL
            TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
     AU 2000028132 A  20000914 (200063)              C12Q001-00
     AU 2000028137 A  20000914 (200063)              G01N033-68
ADT  WO 2000050901 A1 WO 2000-GB668 20000225; AU 2000028132 A AU 2000-28132
     20000225; AU 2000028137 A AU 2000-28137 20000225
FDT  AU 2000028132 A Based on WO 200050630; AU 2000028137 A Based on WO
     200050901
PRAI GB 1999-4395     19990225; GB 1999-4392     19990225; GB 1999-4393
     19990225; GB 1999-4398     19990225; GB 1999-4401     19990225; GB
     1999-4407     19990225; GB 2000-771      20000113
IC   ICM  C12Q001-00; G01N033-68
     ICS  G01N033-543; G01N033-563; G01N033-58



L29        2404 FILE MEDLINE
L30        2350 FILE CAPLUS
L31        1291 FILE BIOSIS
L32        2009 FILE EMBASE
L33          26 FILE WPIDS
L34         278 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L35        8358 L21 AND (LABEL? OR LUMINESCEN? OR FLUORESCEN? OR DOMAIN OR
RADIO
                LABEL? OR HEAVY METAL OR RADIOOPAQUE)

=> s l35 and (fret or fluorescen? resonance energy transfer)

L36          15 FILE MEDLINE
L37          21 FILE CAPLUS
L38          11 FILE BIOSIS
L39          25 FILE EMBASE
L40           2 FILE WPIDS
L41           0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L42          74 L35 AND (FRET OR FLUORESCEN? RESONANCE ENERGY TRANSFER)

=> s l35 and (fcs or fluorescen? correlat? spectroscop?)

L43           0 FILE MEDLINE
L44           0 FILE CAPLUS
L45           0 FILE BIOSIS
L46           0 FILE EMBASE
L47           1 FILE WPIDS
L48           0 FILE JICST-EPLUS
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TOTAL FOR ALL FILES
L49 1 L35 AND (FCS OR FLUORESCEN? CORRELAT? SPECTROSCOP?)

=> d

L49 ANSWER 1 OF 1 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 2000-565475 [52] WPIDS
CR 2000-558404 [48]; 2000-565459 [48]; 2000-572100 [48]; 2000-572119 [48]; 2000-579184 [48]
DNN N2000-417632 DNC C2000-168512
TI Determining the **conformational** state of a **protein**, comprises contacting the **protein** with a **labeled** binding **protein** and assessing the **labeling** of the **protein**.
DC B04 D16 S03
IN COLYER, J; CRAIG, R K
PA (FLUO-N) FLUORESCIENCE LTD
CYC 89
PI WO 2000050901 A1 20000831 (200052)* EN 56p G01N033-68
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
AU 2000028132 A 20000914 (200063) C12Q001-00
AU 2000028137 A 20000914 (200063) G01N033-68
ADT WO 2000050901 A1 WO 2000-GB668 20000225; AU 2000028132 A AU 2000-28132 20000225; AU 2000028137 A AU 2000-28137 20000225
FDT AU 2000028132 A Based on WO 200050630; AU 2000028137 A Based on WO 200050901
PRAI GB 1999-4395 19990225; GB 1999-4392 19990225; GB 1999-4393 19990225; GB 1999-4398 19990225; GB 1999-4401 19990225; GB 1999-4407 19990225; GB 2000-771 20000113
IC ICM C12Q001-00; G01N033-68
ICS G01N033-543; G01N033-563; G01N033-58

=> s l42 and (single chain or scfv)

L50 0 FILE MEDLINE
L51 1 FILE CAPLUS
L52 0 FILE BIOSIS
L53 0 FILE EMBASE
L54 2 FILE WPIDS
L55 0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L56 3 L42 AND (SINGLE CHAIN OR SCFV)

=> s l56 not l49

L57 0 FILE MEDLINE
L58 1 FILE CAPLUS

L59 0 FILE BIOSIS
L60 0 FILE EMBASE
L61 1 FILE WPIDS
L62 0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L63 2 L56 NOT L49

=> dup rem l63

PROCESSING COMPLETED FOR L63
L64 2 DUP REM L63 (0 DUPLICATES REMOVED)

=> d cbib abs 1-2

L64 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2001 ACS
2000:609012 Document No. 133:190185 **Protein** assay for **conformational** state. Colyer, John; Craig, Roger Kingdon (Fluorescience Limited, UK). PCT Int. Appl. WO 2000050901 A1 20000831, 56 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-GB668 20000225. PRIORITY: GB 1999-4395 19990225.

AB The invention relates to a method for detg. the **conformational** state of a **protein**, comprising the steps of: (a) providing a first binding partner which is capable of binding to the **protein** in a manner dependent on the **conformational** state of the **protein** and which generates a signal in a manner dependent on the binding of the first binding partner to the **protein**; and (b) contacting the **protein** with the first binding partner and detg. the **conformational** state of the **protein** by assessing the **labeling** of the **protein** by the binding of the first binding partner.

L64 ANSWER 2 OF 2 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 2000-205772 [18] WPIDS
CR 2000-205672 [17]; 2000-205771 [17]

AB WO 200008444 A UPAB: 20000624
NOVELTY - Method for measuring the degree of **fluorescence** energy **transfer** between a donor and acceptor system using biological constructs, is new.

DETAILED DESCRIPTION - Method (I) for the measurement of the degree of **fluorescence resonance energy transfer** (**FRET**) taking place between a donor (D) and acceptor (A) system comprises:

(1) irradiating a combined donor-acceptor system with a beam of intensity modulated excitation energy of a first wavelength;

(2) receiving **fluorescence** emissions from D and A having overlapping spectra;

(3) simultaneously determining a modulation lifetime (tau mod) and a phase lifetime (tau phi) of the combined emitted **fluorescence** of the D-A system; and

(4) determining a degree of acceptor ingrowth by comparison of tau mod and tau phi .

INDEPENDENT CLAIMS are also included for the following:

(1) a **polypeptide** comprising, in any order a donor chromophore, an acceptor chromophore, a **domain** R comprising an **enzyme** recognition site (ERS) and a **domain** B which either (a) binds to R once the **enzyme** has acted on the ERS, or (b) binds to R when the **enzyme** has not acted on the ERS but does bind once the **enzyme** has acted on the ERS; when B is bound to R and when appropriately irradiated, there is a **change** in the degree of **FRET** between the D and A chromophore compared to when B is not bound to R;

(2) a library of **polypeptides**, each **polypeptide** comprising a D chromophore, an A chromophore, a **domain** B for which it is desired that a **polypeptide** binding partner is identified and a **domain** R which may bind to B so that when appropriately irradiated, there is a **change** in the degree of **FRET** taking place between D and A compared to when R is not bound to B, and where each member of the library has the same D, A and B **domain** but a different R **domain**;

(3) a polynucleotide encoding the **polypeptides** of (1) or (2);

(4) an expression vector encoding the **polypeptides** of (1) or (2); and

(5) a host cell comprising the polynucleotide of (3) or the vector of (4).

USE - The method can be used for determining the relative state populations of a biological system capable of having two states exhibiting different degrees of **FRET** between donor and acceptor molecules (claimed). The **polypeptides**, polynucleotide or host cells can be used in a screening assay to identify a compound which modulates **enzyme activity** (claimed).

The methods are suited to investigating **changes** in biological systems either within a cell (e.g. to test the effect of test compounds on the cell), or in an in vitro system. The methods are used in screening assays for drugs that modulate a biological system in a desirable way. The methods can be applied to directly measure and localize in living cells or organisms, or in vitro, the **activity** of an **enzyme**, and **changes** in concentration of small metabolites or ions. The methods can also be used in genetic screens for phenotypes resulting in **changes** in enzymatic **activity**.

ADVANTAGE - The methods suitable for determining a degree of **fluorescence resonance energy transfer** (**FRET**) in donor-acceptor systems not spectroscopically well-resolved. With respect to intensity or radiometric methods to determine **FRET**, lifetimes are independent of probe concentration, geometries and trivial reabsorption processes; optical filtering is less stringent, allowing collection of almost all of the emitted **fluorescent** light from the acceptor and part of the

donor with a single excitation wavelength; the readout is quantitative and directly related to **FRET** efficiency, and does not require external calibration.

DESCRIPTION OF DRAWING(S) - The figure illustrates a possible **conformational change** in a construct, which can be measured by determining the degree of **fluorescence resonance energy transfer (FRET)** between the two chromophores.

Recognition molecule R

Binding molecule B

Acceptor A

Donor D

Dwg.2/14

```
=> s protein bind? partner?

L65          12 FILE MEDLINE
L66          23 FILE CAPLUS
L67          12 FILE BIOSIS
L68          13 FILE EMBASE
L69          27 FILE WPIDS
L70           0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L71          87 PROTEIN BIND? PARTNER?

=> dis his

     (FILE 'HOME' ENTERED AT 10:24:37 ON 06 FEB 2001)

     FILE 'REGISTRY' ENTERED AT 10:24:47 ON 06 FEB 2001

     FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, WPIDS, JICST-EPLUS' ENTERED AT
     10:25:18 ON 06 FEB 2001
L1        119925 FILE MEDLINE
L2        119805 FILE CAPLUS
L3         57214 FILE BIOSIS
L4         55055 FILE EMBASE
L5          2260 FILE WPIDS
L6         24224 FILE JICST-EPLUS
     TOTAL FOR ALL FILES
L7        378483 S (CONFORMAT? OR CONFIGURAT? OR ARRANGEMENT) AND (PROTEIN OR
PO
L8         63988 FILE MEDLINE
L9         68032 FILE CAPLUS
L10        30949 FILE BIOSIS
L11        34908 FILE EMBASE
L12         1285 FILE WPIDS
L13        10534 FILE JICST-EPLUS
     TOTAL FOR ALL FILES
L14       209696 S L7 AND (BOND? OR HOLD(2A)TOGETHER OR REACTION OR INTERACT?
OR
L15         6592 FILE MEDLINE
```

```
L16         6600 FILE CAPLUS
L17         3429 FILE BIOSIS
L18         4909 FILE EMBASE
L19           49 FILE WPIDS
L20         1487 FILE JICST-EPLUS
     TOTAL FOR ALL FILES
L21        23066 S L14 AND (POST TRANSLAT? OR CHANGE? OR TRANSFER?) AND ENZYME?
L22            0 FILE MEDLINE
L23            0 FILE CAPLUS
L24            0 FILE BIOSIS
L25            0 FILE EMBASE
L26            1 FILE WPIDS
L27            0 FILE JICST-EPLUS
     TOTAL FOR ALL FILES
L28            1 S L21 AND (CAPTURE LIGAND OR SOLID PHASE SUBSTRATE)
L29         2404 FILE MEDLINE
L30         2350 FILE CAPLUS
L31         1291 FILE BIOSIS
L32         2009 FILE EMBASE
L33           26 FILE WPIDS
L34          278 FILE JICST-EPLUS
     TOTAL FOR ALL FILES
L35         8358 S L21 AND (LABEL? OR LUMINESCEN? OR FLUORESCEN? OR DOMAIN OR
RA
L36           15 FILE MEDLINE
L37           21 FILE CAPLUS
L38           11 FILE BIOSIS
L39           25 FILE EMBASE
L40            2 FILE WPIDS
L41            0 FILE JICST-EPLUS
     TOTAL FOR ALL FILES
L42           74 S L35 AND (FRET OR FLUORESCEN? RESONANCE ENERGY TRANSFER)
L43            0 FILE MEDLINE
L44            0 FILE CAPLUS
L45            0 FILE BIOSIS
L46            0 FILE EMBASE
L47            1 FILE WPIDS
L48            0 FILE JICST-EPLUS
     TOTAL FOR ALL FILES
L49            1 S L35 AND (FCS OR FLUORESCEN? CORRELAT? SPECTROSCOP?)
L50            0 FILE MEDLINE
L51            1 FILE CAPLUS
L52            0 FILE BIOSIS
L53            0 FILE EMBASE
L54            2 FILE WPIDS
L55            0 FILE JICST-EPLUS
     TOTAL FOR ALL FILES
L56            3 S L42 AND (SINGLE CHAIN OR SCFV)
L57            0 FILE MEDLINE
L58            1 FILE CAPLUS
L59            0 FILE BIOSIS
L60            0 FILE EMBASE
L61            1 FILE WPIDS
L62            0 FILE JICST-EPLUS
     TOTAL FOR ALL FILES
L63            2 S L56 NOT L49
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L64           2 DUP REM L63 (0 DUPLICATES REMOVED)
L65          12 FILE MEDLINE
L66          23 FILE CAPLUS
L67          12 FILE BIOSIS
L68          13 FILE EMBASE
L69          27 FILE WPIDS
L70           0 FILE JICST-EPLUS
     TOTAL FOR ALL FILES
L71          87 S PROTEIN BIND? PARTNER?

=> s l42 or l71

L72          27 FILE MEDLINE
L73          44 FILE CAPLUS
L74          23 FILE BIOSIS
L75          38 FILE EMBASE
L76          29 FILE WPIDS
L77           0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L78         161 L42 OR L71

=> s l78 not (l49 or l53 or l63)

L79          27 FILE MEDLINE
L80          43 FILE CAPLUS
L81          23 FILE BIOSIS
L82          38 FILE EMBASE
L83          27 FILE WPIDS
L84           0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L85         158 L78 NOT (L49 OR L53 OR L63)

=> dup rem l85

PROCESSING COMPLETED FOR L85
L86          92 DUP REM L85 (66 DUPLICATES REMOVED)

=> s craig r?/au,in;s colyer j?/au,in

'IN' IS NOT A VALID FIELD CODE
L87         725 FILE MEDLINE
L88         753 FILE CAPLUS
L89         770 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE
L90         438 FILE EMBASE
L91          92 FILE WPIDS
L92           3 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L93        2781 CRAIG R?/AU,IN


'IN' IS NOT A VALID FIELD CODE
L94          34 FILE MEDLINE
```

```
L95          49 FILE CAPLUS
L96          65 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE
L97          37 FILE EMBASE
L98           9 FILE WPIDS
L99           0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L100        194 COLYER J?/AU,IN

=> s 193 and l100

L101          0 FILE MEDLINE
L102          5 FILE CAPLUS
L103          0 FILE BIOSIS
L104          0 FILE EMBASE
L105          5 FILE WPIDS
L106          0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L107         10 L93 AND L100

=> dup rem l107

PROCESSING COMPLETED FOR L107
L108          5 DUP REM L107 (5 DUPLICATES REMOVED)

=> d cbib abs 1-5;s 185 and py<=february 1999


L108 ANSWER 1 OF 5  CAPLUS  COPYRIGHT 2001 ACS         DUPLICATE 1
2000:609013   Document No. 133:205085  High throughput assay for protein
     modification.  Colyer, John; Craig, Roger Kingdon; Maschio,
     Antonio; Mezna, Mokdad (Fluorescience Limited, UK).  PCT Int. Appl. WO
     2000050902 A2 20000831, 128 pp.  DESIGNATED STATES: W:  AE, AL, AM, AT,
     AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES,
     FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ,
     LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT,
     RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU,
     ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG,
     CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR,
     NE, NL, PT, SE, SN, TD, TG.  (English).  CODEN: PIXXD2.  APPLICATION: WO
     2000-GB669 20000225.  PRIORITY: GB 1999-4398 19990225.
AB   This invention relates to a method for analyzing a sample comprising:
     immobilizing a polypeptide to a phys. support; contacting the immobilized
     polypeptide with a test sample which may contain an agent capable of
     modifying the immobilized polypeptide; contacting the immobilized
     polypeptide with a binding partner polypeptide, wherein the binding of
     this partner polypeptide to the immobilized polypeptide is at least
partly
     dependent on the modification state of the immobilized polypeptide; and
     measuring the assocn. of the binding partner polypeptide to the
     immobilized polypeptide.  Src kinase assays involved immobilized
     fluorescent natural binding partners that were affected by
     phosphorylation.
```

L108 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 2
2000:609012 Document No. 133:190185 Protein assay for conformational state. **Colyer, John**; Craig, Roger Kingdon (Fluorescience Limited, UK). PCT Int. Appl. WO 2000050901 A1 20000831, 56 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-GB668 20000225. PRIORITY: GB 1999-4395 19990225.

AB The invention relates to a method for detg. the conformational state of a protein, comprising the steps of: (a) providing a first binding partner which is capable of binding to the protein in a manner dependent on the conformational state of the protein and which generates a signal in a manner dependent on the binding of the first binding partner to the protein; and (b) contacting the protein with the first binding partner and detg. the conformational state of the protein by assessing the labeling of the protein by the binding of the first binding partner.

L108 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 3
2000:609007 Document No. 133:190184 Compositions and methods for monitoring the modification of engineered binding partners and for monitoring enzyme activity. **Colyer, John**; Woolfson, Derek; Craig, Roger Kingdon (Fluorescience Limited, UK). PCT Int. Appl. WO 2000050896 A1 20000831, 101 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-GB674 20000225. PRIORITY: GB 1999-4407 19990225.

AB This invention relates to methods and compns. for monitoring enzymic activity as a function of the interaction of binding partners, wherein binding is dependent upon addn. or subtraction of a chem. moiety to or from one of the binding partners by a protein modifying enzyme. Phage displaying mutant ubiquitin fragments were generated and ubiquitin complexes were identified which were upset by PKA phosphorylation.

L108 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 4
2000:608937 Document No. 133:204737 Compositions and methods for monitoring the modification of natural binding partners and enzyme activity. **Colyer, John**; Craig, Roger Kingdon (Fluorescience Limited, UK). PCT Int. Appl. WO 2000050631 A2 20000831, 128 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN:

PIXXD2. APPLICATION: WO 2000-GB666 20000225. PRIORITY: GB 1999-4392 19990225; GB 1999-4393 19990225.

AB This invention relates to methods and compns. for monitoring enzymic activity as a function of the interaction of binding partners, wherein binding is dependent upon addn. or subtraction of a chem. moiety to or from one of the binding partners by a protein modifying enzyme. Thus, a soln. phase FRET assay for Yersinia tyrosine phosphatase was described. The binding partners used were rhodamine-labeled, phosphorylated TCR.zeta. chain fragment and the SH2 domain of ZAP-70 fused to GFP. This same assay was used to det. the IC50 for orthovanadate for this enzyme.

L108 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 5

2000:608936 Document No. 133:204736 Assay for measuring different enzyme activities simultaneously. **Colyer, John**; Craig, Roger Kingdon (Fluorescience Limited, UK). PCT Int. Appl. WO 2000050630 A2 20000831, 78 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-GB663 20000225. PRIORITY: GB 1999-4401 19990225; GB 1999-4392 19990225; GB 1999-4393 19990225; GB 1999-4398 19990225; GB 1999-4395 19990225; GB 1999-4407 19990225; GB 2000-771 20000113.

AB A method is provided for measuring simultaneously the activity of a first enzyme and a second enzyme in a system which method comprises: (a) contacting a first binding domain and a first binding partner thereof with said first enzyme and contacting a second binding domain and a second binding partner thereof with said second enzyme; wherein (i) the first binding domain and/or binding partner comprise a site subject to post-translational modification by the first enzyme; (ii) modification of the site by the first enzyme affects the interaction between the first binding partner; (iii) the second binding domain and/or binding partner comprise a site subject to post-translational modification by the second enzyme; and (iv) modification of the site by the second enzyme affects the interaction between the second binding domain and second binding partner; and (b) measuring the interaction between the first binding domain and the first binding partner and measuring the interaction between the second binding domain and the second binding partner. Thus, fluorescence polarization assays for simultaneously measuring protein kinase A and TEV protease activities, or protein kinase A and calmodulin-dependent protein kinase II activities, were demonstrated. The substrates for the various enzymes were coiled-coil peptides labeled with coumarin or biotin for one enzyme, or with fluorescein or biotin for the second.

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L109 ANSWER 1 OF 28 CAPLUS COPYRIGHT 2001 ACS
1999:701347 Document No. 132:89652 Arf proteins bind to mitotic kinesin-like protein 1 (MKLP1) in a GTP-dependent fashion. Boman, Annette L.; Kuai, Jun; Zhu, Xinjun; Chen, Jing; Kuriyama, Ryoko; Kahn, Richard A. (Department of Biochemistry, Emory University School of Medicine, Atlanta, GA, USA). Cell Motil. Cytoskeleton, 44(2), 119-132 (English) **1999**. CODEN: CMCYEO. ISSN: 0886-1544. Publisher: Wiley-Liss, Inc..
AB Arf proteins comprise a family of 21-kDa GTP-binding proteins with many proposed functions in mammalian cells, including the regulation of several steps of membrane transport, maintenance of organelle integrity, and activation of phospholipase D. We performed a yeast two-hybrid screen of human cDNA libraries using a dominant activating allele, [Q71L], of human Arf3 as bait. Eleven independent isolates contained plasmids encoding the C-terminal tail of mitotic kinesin-like protein-1 (MKLP1). Further deletion mapping allowed the identification of an 88 amino acid Arf3 binding domain in the C-terminus of MKLP1. This domain has no clear homol. to other Arf binding proteins or to other proteins in the protein databases. The C-terminal domain of MKLP1 was expressed and purified from bacteria as a GST fusion protein and shown to bind Arf3 in a GTP-dependent fashion. A screen for mutations in Arf3 that specifically lost the ability to bind MKLP1 identified 10 of 14 point mutations in the GTP-sensitive switch I or switch II regions of Arf3. Two-hybrid assays of the C-terminal domain of MKLP1 with each of the human Arf isoforms revealed strong interaction with each. Taken together, these data are all supportive of the conclusion that activated Arf proteins bind to the C-terminal tail domain of MKLP1.

L109 ANSWER 2 OF 28 CAPLUS COPYRIGHT 2001 ACS
1999:651395 Document No. 132:1386 Identification and characterization of polycystin-2, the PKD2 gene product. Cai, Yiqiang; Maeda, Yoshiko; Cedzich, Anna; Torres, Vicente E.; Wu, Guanqing; Hayashi, Tomohito; Mochizuki, Toshio; Park, Jong Hoon; Witzgall, Ralph; Somlo, Stefan (Renal Division, Departments of Medicine and Molecular Genetics, Albert Einstein College of Medicine, Bronx, NY, 10461, USA). J. Biol. Chem., 274(40), 28557-28565 (English) **1999**. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB PKD2, the second gene for the autosomal dominant polycystic kidney disease (ADPKD), encodes a protein, polycystin-2, with predicted structural similarity to cation channel subunits. However, the function of polycystin-2 remains unknown. The authors used polyclonal antisera specific for the intracellular NH2 and COOH termini to identify polycystin-2 as an .apprx.110-kDa integral membrane glycoprotein. Polycystin-2 from both native tissues and cells in culture is sensitive to Endo H suggesting the continued presence of high-mannose oligosaccharides typical of pre-middle Golgi proteins. Immunofluorescent cell staining of polycystin-2 shows a pattern consistent with localization in the endoplasmic reticulum. This finding is confirmed by co-localization with protein-disulfide isomerase as detd. by double indirect immunofluorescence and co-distribution with calnexin in subcellular fractionation studies. Polycystin-2 translation products truncated at or after Gly821 retain their exclusive endoplasmic reticulum localization while products truncated at or before Glu787 addnl. traffic to the plasma membrane. Truncation mutants that traffic to the plasma membrane acquire Endo H resistance and can be biotinylated on the cell surface in intact cells. The 34-amino acid region Glu787-Ser820, contg. two putative phosphorylation sites, is responsible for the exclusive endoplasmic reticulum localization of polycystin-2 and is the site of specific interaction with an as yet unidentified **protein binding partner** for polycystin-2. The localization of full-length polycystin-2 to intracellular membranes raises the possibility that the PKD2 gene product is a subunit of intracellular channel complexes.

L109 ANSWER 3 OF 28 CAPLUS COPYRIGHT 2001 ACS

1999:615340 Document No. 131:319008 The PTB domain: the name doesn't say it all. Margolis, Ben (Howard Hughes Medical Institute, Departments of Internal Medicine and Biological Chemistry, University of Michigan Medical School, Ann Arbor, MI, 48109-0650, USA). Trends Endocrinol. Metab., 10(7), 262-267 (English) **1999**. CODEN: TENME4. ISSN: 1043-2760. Publisher: Elsevier Science Ltd..

AB A review with 57 refs. The phosphotyrosine-binding (PTB) domain is a recently described protein-protein interaction domain which, despite its name, is involved in both phosphotyrosine-dependent and -independent interactions. Proteins with this domain are involved in diverse cellular functions, ranging from receptor signaling to protein targeting.

L109 ANSWER 4 OF 28 CAPLUS COPYRIGHT 2001 ACS

1999:568397 Document No. 131:320490 Emerging roles for RGS proteins in cell signalling. Hepler, J. R. (5009 Rollins Research Center, Department of Pharmacology, Emory University School of Medicine, Atlanta, GA, USA). Trends Pharmacol. Sci., 20(9), 376-382 (English) **1999**. CODEN: TPHSDY. ISSN: 0165-6147. Publisher: Elsevier Science Ltd..

AB A review with 84 refs. Regulators of G-protein signalling (RGS proteins) are a family of highly diverse, multifunctional signalling proteins that share a conserved 120 amino acid domain (RGS domain). RGS domains bind directly to activated G.alpha. subunits and act as GTPase-activating proteins (GAPs) to attenuate and/or modulate hormone and neurotransmitter receptor-initiated signalling by both G.alpha.-GTP and G.beta..gamma.. Apart from this structural domain, which is shared by all known RGS

proteins, these proteins differ widely in their overall size and amino acid identity and possess a remarkable variety of structural domains and motifs. These biochem. features impart signalling functions and/or enable RGS proteins to interact with a growing list of unexpected **protein -binding partners** with diverse cellular roles. New appreciation for the broader cellular functions of RGS proteins challenges established models of G-protein signalling and serves to identify these proteins as central participants in receptor signalling and cell physiol.

L109 ANSWER 5 OF 28 CAPLUS COPYRIGHT 2001 ACS
1999:543212 Observing single biomolecule **reactions**.. Weiss, Shimon; Dahan, Maxime; Lacoste, Thilo; Glass, Jennifer; Laurence, Ted; Chemla, Daniel S.; Deniz, Ashok; Ting, Alice; Grunwell, Jocelyn; Faulhaber, Ann E.; Schultz, Peter G. (Materials Sciences and Physical Biosciencs Divisions, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720, USA). Book of Abstracts, 218th ACS National Meeting, New Orleans, Aug. 22-26, PHYS-080. American Chemical Society: Washington, D. C. (English) **1999**. CODEN: 67ZJA5.
AB We developed single mol. **fluorescence** spectroscopy tools that can relay the **conformational** dynamics of single biol. mols. to the phys. observables. **Fluorescence resonance energy transfer** (**FRET**),which measures the proximity between two fluorophores, and **fluorescence** polarization anisotropy (FPA), which reports on the fluorophore's rotational dynamics, can provide information on **activity**, function and local structural **changes**. Single mol. methods have important advantages over conventional ensemble measurements: (1) they can resolve and quant. compare distinct sub-populations of **conformational** states, otherwise invisible at the ensemble level; (2) they can resolve fluctuations and dynamic **conformational changes**.The ability to identify **conformational** states and separatesub-populations of individual macromols. freely diffusing in a heterogeneous soln. is demonstrated .Single mol. studies of single immobilized **enzyme** mols. reveal millisecond distance fluctuations andmeasure single catalytic events. Applications to singlemol. enzymol. and **protein** folding will be discussed.

L109 ANSWER 6 OF 28 CAPLUS COPYRIGHT 2001 ACS
1999:348943 Document No. 131:155184 **Interactions** of **fluorescent** triacylglycerol analogs covalently bound to the active site of a lipase from Rhizopus oryzae. Zandonella, Gerhild; Stadler, Peter; Haalck, Lutz; Spener, Fritz; Paltauf, Fritz; Hermetter, Albin (Department of Biochemistry and Food Chemistry, Technische Universitat, Graz, A-8010, Austria). Eur. J. Biochem., 262(1), 63-69 (English) **1999**. CODEN: EJBCAI. ISSN: 0014-2956. Publisher: Blackwell Science Ltd..
AB **Fluorescent** triacylglycerol analogs were synthesized as covalent inhibitors of lipase **activity**. The resp. 1(3),2-O-dialkylglycero-3(1)-alkyl-phosphonic acid p-nitrophenyl esters contain a **fluorescent** pyrenealkyl chain and a long-chain alkyl residue bound to the sn-2 and sn-1(3) positions of glycerol, resp. The phosphonic acid p-nitrophenyl ester **bond** is susceptible to nucleophilic

substitution by the active serine residue in the catalytic triad of a lipase, leading to inactivation of the **enzyme**. The **fluorescent** dialkylglycerophosphonates contain two chiral centers, the sn-2 carbon of glycerol and the phosphorus atom. The (1-O-hexadecyl-2-O-pyrenedecyl-sn-glycero)-O-(p-nitrophenyl)-n-hexyl-phosphonate, first peak during HPLC sepn. and the (3-O-hexadecyl-2-O-pyrenedecyl-sn-glycero)-O-(p-nitrophenyl)-n-hexyl-phosphonate, second peak during HPLC sepn. were found to be potent lipase inhibitors. After incubation of an equimolar amt. of these isomers with lipase from Rhizopus oryzae complete inactivation was obsd. Stable conjugates contg. a 1: 1 molar ratio of lipid to **protein** were formed. The spatial proximity of the **fluorescently labeled** sn-2 alkyl chain of the inhibitor and tryptophan residues of the lipase was assessed by **fluorescence resonance energy transfer**. The extent of tryptophan **fluorescence** quenching and the concomitant increase in pyrene **fluorescence** upon excitation of lipase tryptophans was found to be similar for the above-mentioned isomers. Thus, the (**labeled**) sn-2 alkyl chains of a triacylglycerol analog are likely to **interact** with the same binding site of the R. oryzae lipase, irresp. of their steric **configuration**. However, it was shown that the extent of resonance energy **transfer** is strongly influenced by the **reaction** medium, indicating **conformational changes** of the lipase in different environments.

L109 ANSWER 7 OF 28 CAPLUS COPYRIGHT 2001 ACS
1999:284659 Document No. 131:114054 Regulatory role of SH3 domain-mediated protein-protein interactions in synaptic vesicle endocytosis. McPherson, Peter S. (Department of Neurology and Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, PQ, H3A 2B4, Can.). Cell. Signalling, 11(4), 229-238 (English) **1999**. CODEN: CESIEY. ISSN: 0898-6568. Publisher: Elsevier Science Inc..
AB A review with 127 refs. Src homol. (SH) 3 domains are small modules found in a diverse array of proteins. The presence of an SH3 domain confers upon its resident protein the ability to interact with specific proline-rich sequences in **protein binding partners**. A major focus of research has highlighted a role for SH3 domain-mediated interactions in the regulation of signal transduction events. However, more recent data has suggested an important function for SH3 domains in vesicular trafficking. This review will focus on this newly emerging role with a particular emphasis on the mol. components involved in synaptic vesicle endocytosis and the regulatory role of SH3 domain-mediated protein-protein interactions in this process.

L109 ANSWER 8 OF 28 CAPLUS COPYRIGHT 2001 ACS
1999:99196 Document No. 130:248720 Single-molecule **fluorescence** spectroscopy of **enzyme conformational** dynamics and cleavage mechanism. Ha, Taekjip; Ting, Alice Y.; Liang, Joy; Caldwell, W. Brett; Deniz, Ashok A.; Chemla, Daniel S.; Schultz, Peter G.; Weiss, Shimon (Materials Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720, USA). Proc. Natl. Acad. Sci. U. S. A.,

96(3), 893-898 (English) **1999**. CODEN: PNASA6. ISSN: 0027-8424. Publisher: National Academy of Sciences.

AB **Fluorescence resonance energy transfer** and **fluorescence** polarization anisotropy were used to investigate single mols. of staphylococcal nuclease (I). Intramol. **fluorescence resonance energy transfer** and **fluorescence** polarization anisotropy measurements of **fluorescently labeled** I mols. revealed distinct patterns of fluctuations that may be attributed to **protein conformational** dynamics on the millisecond time scale. Intermol. **fluorescence resonance energy transfer** measurements provided information about the dynamic **interactions** of I with single substrate mols. The exptl. methods demonstrated here should prove generally useful in studies of **protein** folding and **enzyme** catalysis at single-mol. resoln.

L109 ANSWER 9 OF 28 CAPLUS COPYRIGHT 2001 ACS

1998:711606 Document No. 130:49150 Aflatoxin B1 and sulfobromophthalein binding to the dimeric human glutathione S-**transferase** A1-1: a **fluorescence** spectroscopic analysis. Sluis-Cremer, Nicolas; Wallace, Louise; Burke, Jonathan; Stevens, Julie; Dirr, Heini (Protein Structure-Function Research Programme, Department of Biochemistry, University of the Witwatersrand, Johannesburg, 2050, S. Afr.). Eur. J. Biochem., 257(2), 434-442 (English) **1998**. CODEN: EJBCAI. ISSN: 0014-2956. Publisher: Springer-Verlag.

AB The binding **interactions** between dimeric human class alpha glutathione S-**transferase** A1-1 (GST A1-1) and aflatoxin B1 or sulfobromophthalein (BSP) were characterized. Aflatoxin B1 binds to GST A1-1 with a stoichiometry of 1.1 mol/mol of dimeric **enzyme**. The binding **interaction**, which can be described by a hyperbolic satn. isotherm (Kd = 8 .+-. 2 .mu.M), does not induce major structural **changes** in the **enzyme**, nor does it inhibit enzymic **activity**. The av. distance between the single tryptophan residue (Trp20) of GST A1-1 and **protein**-bound aflatoxin B1 was calcd. to be 22.7 .ANG. by means of **fluorescence resonance energy transfer**. The aflatoxin-binding region, according to this calcd. distance, was detd. to be located in the dimer interface cleft near the crystallog. two-fold axis. Hill-plot analyses suggest that a pos. co-operative **interaction** exists between BSP and the dimeric GST A1-1 (h = 1.6 .+-. 0.1; K' = 14 .+-. 0.6 .mu.M). The binding of BSP induces a **conformational change** in the **enzyme** which is accompanied by a decrease in the mol. flexibility and in the solvent-accessible properties of the **enzyme**'s Trp20 residue. Site-directed mutagenesis of Trp20 (Trp20.fwdarw.Phe) confirms that this residue is situated in the binding environment and although it is not essential for BSP binding, it is involved in the **interaction**. Furthermore, the structural **change** assocd. with BSP binding alters the hyperbolic character of the glutathione satn. curve. This indicates that there may also be a cooperative **interaction** between glutathione and BSP or that BSP binding induces asym. functioning of the two **enzyme** subunits so that they become unequal in catalytic **activity**.

L109 ANSWER 10 OF 28 CAPLUS COPYRIGHT 2001 ACS

1998:636306 Document No. 130:11611 Regulation of the Rate and Extent of

Phospholipase C .beta.2 Effector Activation by the .beta..gamma. Subunits of Heterotrimeric G **Proteins**. Runnels, Loren W.; Scarlata, Suzanne F. (Department of Physiology Biophysics, State University of New York at Stony Brook, Stony Brook, NY, 11794-8661, USA). Biochemistry, 37(44), 15563-15574 (English) **1998**. CODEN: BICHAW. ISSN: 0006-2960. Publisher: American Chemical Society.

AB The **activity** of mammalian phosphoinositide-specific phospholipase C .beta.2 (PLC-.beta.2) is regulated by the .alpha.q family of G **proteins** and by .beta..gamma. subunits. We measured the affinity between the laterally assocg. PLC-.beta.2 and G.beta..gamma. on membrane surfaces by **fluorescence resonance energy transfer**. Using a simple model, we translated this apparent affinity to a bulk or three-dimensional equil. const. (Kd) and obtained a value of 3.2 .mu.M. We confirmed this Kd by sep. measuring the on and off (kf and kr) rate consts. The kf was slower than a diffusion-limited value, suggesting that **conformational changes** occur when the two **proteins interact**. The off rate shows that the PLC-.beta.2.cntdot.G.beta..gamma. complexes are long-lived (.apprx.123 s) and that activation of PLC-.beta.2 by G.beta..gamma. would be sustained without a deactivating factor. The addn. of .alpha.i1(GDP) subunits failed to phys. dissoc. the complex as detd. by **fluorescence**. However, **enzyme activity** studies performed under similar conditions show that the addn. of G.alpha.i1(GDP) results in reversal of PLC-.beta.2 activation by G.beta..gamma. during the time of the assay (30 s). From these results, we propose that G.alpha.(GDP) subunits can bind to the PLC-.beta.2.cntdot.G.beta..gamma. complex to allow for rapid deactivation without complex dissocn. In support of this model, we show by **fluorescence** that G.alpha.i1(GDP).cntdot.G.beta..gamma..cntdot.PLC-.beta.2 can form.

L109 ANSWER 11 OF 28 CAPLUS COPYRIGHT 2001 ACS

1998:257733 Document No. 128:266733 Rapid screening for protein interactors using in vitro-translated protein and an expression library. Robert, Stanley; Saint, Robert (Dep. of Genetics, Univ. of Adelaide, 5005, Australia). Tech. Tips Online No pp. Given (English) **1998**. CODEN: TTONFG. URL: http://tto.trends.com/cgi-bin/tto/pr/pg_art.cgi?sid=art.new&ac=t01334|/cgi-bin/tto/pr/pg_new.cgi Publisher: Elsevier Trends Journals.

AB A simple and rapid system was developed for detecting **protein binding partners** that does not relay on a transcriptional assay, as in the yeast two-hybrid system, but still provides a cDNA clone encoding the interacting protein. The method involves expression of proteins from a .lambda.GT11 cDNA library plated on a bacterial lawn and transfer of the resulting proteins to nitrocellulose membranes using a modification of the conventional plaque lift technique. These membranes are than probes with radiolabeled in vitro translated bait protein and the filters washed and autoradiographed. The use of radiolabeled in vitro-translated protein obviates the requirement for complex purifn. procedures, antibodies against the protein bait or post-translational labeling.

L109 ANSWER 12 OF 28 CAPLUS COPYRIGHT 2001 ACS

1997:574467 Document No. 127:202553 Method for characterization of the fine structure of protein binding sites. Woods, Virgil L., Jr. (Regents of the University of California, USA). U.S. US 5658739 A **19970819**, 28 pp. (English). CODEN: USXXAM. APPLICATION: US 1994-240593 19940510.

AB The binding sites of binding proteins and their binding partners are characterized, at the individual amino acid level, by a combination of tritium exchange labeling and sequential degrdn. and anal. of tritiated fragments under slowed exchange conditions. The invention provides substantially higher resoln. of the sites of functional tritium labeling and provides a method for the functional labeling of specific amino acid residues that participate in binding **protein-binding partner** interactions. It is esp. suitable for the study of the binding **protein-binding partner** subregions of large (>30 kilodaltons) proteins, even in small quantities. As an example, the author studied the interaction of human Hb with 2 different monoclonal antibodies known to be reactive with defined and previously identified subregions of the Hb-binding protein haptoglobin. A variation of the invention uses deuterium exchange labeling.

L109 ANSWER 13 OF 28 CAPLUS COPYRIGHT 2001 ACS

1997:549401 Document No. 127:231137 Design and characterization of a multisite **fluorescence** energy-**transfer** system for **protein** folding studies: A steady-state and time-resolved study of yeast phosphoglycerate kinase. Lillo, M. Pilar; Beechem, Joseph M.; Szpikowska, Barbara K.; Sherman, Mark A.; Mas, Maria T. (Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN, 37232, USA). Biochemistry, 36(37), 11261-11272 (English) **1997**. CODEN: BICHAW. ISSN: 0006-2960. Publisher: American Chemical Society.

AB A multisite distance-based **fluorescence resonance energy-transfer** assay system was developed for the study of **protein** folding **reactions**. Single- and double-cysteine substitution mutagenesis was utilized to place sulfhydryl residues throughout the tertiary structure of the bi-**domain enzyme** yeast phosphoglycerate kinase (PGK). These reactive cysteines were covalently modified with extrinsic donor [5-[[2-(2-iodoacetamido)ethyl]amino]-1-naphthalenesulfonic acid] and acceptor (5-iodoacetamidofluorescein) **fluorescent labels**. A detailed exptl. strategy was followed, which revealed that, when these relatively large extrinsic **fluorescent labels** are covalently attached to properly selected solvent-exposed residues, they do not affect the intrinsic stability of the **protein**. The PGK crystal structure was combined with mol. dynamics simulations of the dyes built into the **protein** and time-resolved anisotropy expts., in order to est. a more realistic orientation factor, .ltbbrac..kappa.2.rtbbrac.*, for each donor/acceptor pair. Time-resolved and steady-state **fluorescence** energy-**transfer** expts. revealed that this distance assay, spanning six different donor-acceptor distances, is linear and accurate (to within 10-20%) over the range of 30-70 .ANG.. This distance assay system for PGK allows for the measurement of long-range **changes** in intra- and interdomain spatial organization during **protein** folding **reactions**. The approach which we have developed can be applied to any **protein** system in which unique one- and two-site cysteine residues can be engineered into a **protein**. In the following paper [Lillo, M.

P., et al. (1997) Biochem. 36, XXXXX-XXXXX], these multisite energy-**transfer** pairs are utilized for stopped-flow unfolding studies.

L109 ANSWER 14 OF 28 CAPLUS COPYRIGHT 2001 ACS
1997:536206 Document No. 127:244436 Molecular characterization of abLIM, a novel actin-binding and double zinc finger protein. Roof, Dorothy J.; Hayes, Annmarie; Adamian, Michael; Chishti, Athar H.; Li, Tiansen (Berman-Gund Laboratory for the Study of Retinal Degenerations, Department of Ophthalmology, Harvard Medical School, Boston, MA, 02114, USA). J. Cell Biol., 138(3), 575-588 (English) **1997**. CODEN: JCLBA3. ISSN: 0021-9525. Publisher: Rockefeller University Press.
AB Mols. that couple the actin-based cytoskeleton to intracellular signaling pathways are central to the processes of cellular morphogenesis and differentiation. We have characterized a novel protein, the actin-binding LIM (abLIM) protein, which could mediate such interactions between actin filaments and cytoplasmic targets. AbLIM protein consists of a COOH-terminal cytoskeletal domain that is fused to an NH2-terminal domain consisting of four double zinc finger motifs. The cytoskeletal domain is .apprx.50% identical to erythrocyte dematin, an actin-bundling protein of the red cell membrane skeleton, while the zinc finger domains conform to the LIM motif consensus sequence. In vitro expression studies demonstrate that abLIM protein can bind to F-actin through the dematin-like domain. Transcripts corresponding to three distinct isoforms have a widespread tissue distribution. However, a polypeptide corresponding to the full-length isoform is found exclusively in the retina and is enriched in biochem. exts. of retinal rod inner segments. AbLIM protein also undergoes extensive phosphorylation in light-adapted retinas in vivo, and its developmental expression in the retina coincides with the elaboration of photoreceptor inner and outer segments. Based on the composite primary structure of abLIM protein, actin-binding capacity, potential regulation via phosphorylation, and isoform expression pattern, we speculate that abLIM may play a general role in bridging the actin-based cytoskeleton with an array of potential LIM **protein-binding partners**. The developmental time course of abLIM expression in the retina suggests that the retina-specific isoform may have a specialized role in the development or elaboration of photoreceptor inner and outer segments.

L109 ANSWER 15 OF 28 CAPLUS COPYRIGHT 2001 ACS
1997:404609 Document No. 127:75654 Sensitization of cancer cells treated with cytotoxic drugs to Fas-mediated cytotoxicity. Micheau, Olivier; Solary, Eric; Hammann, Arlette; Martin, Francois; Dimanche-Boitrel, Marie-Therese (Unite de Formation et de Recherche de Medecine, Contrat Jeune Formation de I'Institut National de la Sante et de la Recherche Medicale (INSERM) 94-08, Dijon, 21033, Fr.). J. Natl. Cancer Inst., 89(11), 783-789 (English) **1997**. CODEN: JNCIEQ. ISSN: 0027-8874. Publisher: Oxford University Press.
AB The transmembrane receptor Fas, together with its **protein-binding partner** (Fas ligand), is a key regulator of programmed cell death (i.e., apoptosis). Fas and Fas ligand also influence the ability of cytotoxic T lymphocytes and natural killer cells to eliminate tumor cells. However, by inducing apoptosis in activated T

cells, the Fas/Fas ligand system may protect some tumor cells from clearance by the immune system. Anticancer drugs enhance Fas ligand expression on the surface of Fas receptor-expressing leukemia cells, thus suggesting that apoptosis caused by these drugs may be mediated via the Fas/Fas ligand system. This study was conducted to further investigate the relationship between the modulation of Fas receptor gene and protein expression by treatment of cells with cytotoxic drugs and the immune clearance of tumor cells. Fas expression on human HT29 colon carcinoma cells treated with a variety of anticancer drugs (cisplatin, doxorubicin, mitomycin C, fluorouracil, and camptothecin) was analyzed by use of quant. flow cytometry. Human HCT8R and HCT116 colon carcinoma cells and human U937 leukemia cells were treated with cisplatin only and analyzed in the same way. Fas ligand mRNA and protein levels were studied by use of a reverse transcription-polymerase chain reaction assay and by flow cytometry. Fas gene expression and mRNA levels in cisplatin-treated HT29 cells were characterized by use of in vitro nuclear run-on and northern blot hybridization assays. The cytotoxic activities of agonistic anti-Fas antibodies, Fas ligand, and allogeneic peripheral blood leukocytes, in the absence or presence of Fas-blocking monoclonal antibodies, against tumor cells were assessed by methylene blue staining and chromium-51 release assays. Clin. relevant concns. of cisplatin, doxorubicin, mitomycin C, fluorouracil, or camptothecin enhanced Fas receptor expression on the plasma membrane of HT29 cells. Cisplatin-mediated increases in Fas expression were confirmed in HCT8R, HCT116, and U937 cells. The enhancement of Fas protein expression was assocd. with an increased sensitivity of cisplatin-treated tumor cells to agonistic anti-Fas antibodies, to sol. Fas ligand, and to allogeneic peripheral blood leukocyte-mediated cytotoxicity. Each of these effects was blocked by co-treatment of the cells with antagonistic anti-Fas antibody. In addn. to their direct cytotoxic effects, chemotherapeutic drugs sensitize tumor cells to Fas-mediated cytotoxicity and Fas-dependent immune clearance. On the basis of these findings, new strategies might be developed to improve the efficacy of these drugs.

L109 ANSWER 16 OF 28 CAPLUS COPYRIGHT 2001 ACS

1996:687133 Document No. 125:321533 Refolding of triosephosphate isomerase in low-water media investigated by **fluorescence resonance energy transfer**. Sepulveda-Becerra, M. A.; Ferreira, S. T.; Strasser, R. J.; Garzon-Rodriguez, W.; Beltran, C.; Gomez-Puyou, A.; Darszon, A. (Instituto de Biotecnologia, Universidad Nacional Autonoma de Mexico, Morelos, 62271, Mex.). Biochemistry, 35(49), 15915-15922 (English) **1996**. CODEN: BICHAW. ISSN: 0006-2960.

AB The refolding and reassocn. of rabbit muscle triosephosphate isomerase (TPI) monomers unfolded with guanidine-HCl (GdnHCl) were studied in aq. media and in reverse micelles (RM) formed with hexadecyltrimethylammonium bromide and n-octane/hexanol. **Fluorescence resonance energy transfer** (**FRET**) studies were carried out using TPI **labeled** at Cys-217 with 5-[(2-((iodoacetyl)amino)ethyl)amino]naphthalene-1-sulfonic acid (1,5-IAEDANS) and TPI **labeled** at 1 of the free amino groups with fluorescein 5'-isothiocyanate (FITC). Efficient **FRET** between monomers of

AEDANS-TPI and FITC-TPI in aq. media was detected 2-3 min after denaturant diln. and remained const. for hours. The distance between AEDANS and FITC in a **labeled**, renatured hetero-TPI dimer calcd. from **FRET** results was 48 .ANG., in reasonable agreement with ests. based on the crystal structure of TPI. In RM, recovery of **enzyme activity** during renaturation correlated with the appearance of a high-intrinsic **fluorescence** intermediate believed to be a partially folded monomer. Nevertheless, when AEDANS- and FITC-**labeled** monomers were mixed in RM, **FRET** occurred as soon as GdnHCl was dild. (**FRET** efficiency = 0.36), preceding the **changes** in TPI intrinsic **fluorescence** and reactivation. Thereafter, the efficiency of **FRET** increased during the next hour up to .apprx.0.50, where it remained after 24 h, when 80% of the **enzyme activity** was recovered. The high initial **FRET** seen in RM could indicate the formation of an inactive dimer within the 1st minutes after denaturant diln. The further increase in **FRET** obsd. over the next hour could reflect **conformational** rearrangements of the **protein** and transition from the inactive to the active dimer.

L109 ANSWER 17 OF 28 CAPLUS COPYRIGHT 2001 ACS

1996:473265 Document No. 125:106929 Scaffold/Matrix-Attached Regions Act upon Transcription in a Context-Dependent Manner. Schuebeler, Dirk; Mielke, Christian; Maass, Karin; Bode, Juergen (Gesellschaft fuer Biotechnologische Forschung mbH, Braunschweig, D-38124, Germany). Biochemistry, 35(34), 11160-11169 (English) **1996**. CODEN: BICHAW. ISSN: 0006-2960.

AB Scaffold/matrix-attached regions (S/MARs) are cis-acting elements with a function outside transcribed regions and in introns. Although they usually augment transcriptional rates, their action is highly context-dependent. We cloned an 800 bp S/MAR element from the upstream border of the human interferon-.beta. domain at various positions within a transcribed region of 4.3 kb. By use of retroviral gene transfer, the vector could be integrated into target cells as a single copy enabling a rigorous definition of the distance between the S/MAR and the transcriptional start site. At a distance of about 4 kb, the S/MAR supported transcriptional initiation, whereas at distances below 2.5 kb, transcription was essentially shut off. Controls proved the functionality of all constructs in the transient expression phase and ruled out any influence of S/MAR position on transcript stability. Moreover, no pausing or premature termination was obsd. within these elements. We suggest that the **protein binding partners** of S/MARs change according to the topol. status, explaining these divergent S/MAR effects.

L109 ANSWER 18 OF 28 CAPLUS COPYRIGHT 2001 ACS

1993:642809 Document No. 119:242809 v-Myc, but not Max, possesses domains that function in both transcription activation and cellular transformation. Min, Soyoung; Taparowsky, Elizabeth J. (Dep. Biol. Sci., Purdue Univ., West Lafayette, IN, 47907, USA). Oncogene, 7(8), 1531-40

(English) **1992**. CODEN: ONCNES. ISSN: 0950-9232.

AB Deregulated expression of myc gene family members is assocd. with the development of malignant neoplasms in several species. Despite the evidence linking expression of this family of nuclear proteins with the proper control of cellular growth and development, the function of the myc protein remains unknown. Intrigued by the obsd. structural similarity between the myc protein and several eukaryotic transcription factors, the authors have investigated the ability of the MC29 viral myc protein to activate transcription of a heterologous promoter in C3H10T1/2 cells. Overlapping portions of v-myc coding sequences were inserted 3' to the yeast GAL4 DNA-binding domain and tested for their ability to activate transcription of a chloramphenicol acetyl transferase reporter gene contg. GAL4 binding sites. Two transcription activation domains were identified within the amino terminus of v-Myc. The importance of these regions for cellular transformation was examd. using ras/myc co-transformation assays. The authors' results demonstrate that deletion of either of the transcription activation domains, or the DNA-binding and protein oligomerization domains, abolishes the ability of v-Myc to cooperate with Ras to transform C3H10T1/2 cells. Similarly, the authors investigated whether Max, the **protein-binding partner** of Myc, also possesses the potential to activate transcription. Interestingly, chimeric GAL4/Max proteins were not functional in the authors' assays, suggesting that the potential of the Myc-Max complex to influence gene expression and function in cellular transformation relies primarily on sequences found within the amino terminus of Myc.

L109 ANSWER 19 OF 28 CAPLUS COPYRIGHT 2001 ACS

1993:576603 Document No. 119:176603 Functional nucleotide-binding **domain** in the F0F1-ATP synthase .alpha. subunit from the yeast Schizosaccharomyces pombe. Falson, Pierre; Penin, Francois; Divita, Gilles; Lavergne, Jean Pierre; Di Pietro, Attilio; Goody, Roger S.; Gautheron, Daniele C. (Lab. Biochim., Ec. Polytech., Palaiseau, 91128, Fr.). Biochemistry, 32(39), 10387-97 (English) **1993**. CODEN: BICHAW. ISSN: 0006-2960.

AB The segment R165-T330 of the .alpha. subunit of Schizosaccharomyces pombe F1-ATPase, corresponding to a putative nucleotide-binding **domain** by comparison with related nucleotide-binding **proteins**, has been overexpressed in Escherichia coli. Produced as a nonsol. material, it was purified in a nonnative form, using a rapid procedure that includes one reversed-phase chromatog. step. Refolding of the **domain**, called DN.alpha.19, was achieved quant. by using a high-diln. step and monitored by CD and intrinsic **fluorescence**. Once folded, DN.alpha.19 was highly sol. and stable. It bound 1 mol/mol either of adenine or guanine di- or triphosphate nucleotide, with a Kd ranging from 2.3 to 5.4 .mu.M, or of methylanthraniloyl derivs. of the same nucleotides, with a Kd ranging from 0.2 to 0.6 .mu.M. Interestingly, DN.alpha.19 was able to hydrolyze nucleoside triphosphates at a low but significant rate. The distance between one tryptophan residue located in the nucleotide-binding site and the ribose-linked methylanthraniloyl group of di- or triphosphate nucleotides was estd. by **fluorescence resonance energy transfer** to be 13 or 11 .ANG., resp., suggesting

that the tryptophan is close to the polyphosphate moiety of the nucleotide. This tryptophan residue was tentatively assigned to W190 by a hydrophobic cluster comparison with the H-ras p21 **protein**, suggesting that the putative loop of DN.alpha.19 contg. W190 could play a function role in nucleotide binding.

L109 ANSWER 20 OF 28 CAPLUS COPYRIGHT 2001 ACS
1993:444033 Document No. 119:44033 **Fluorescence resonance energy transfer** within a heterochromatic cAMP-dependent **protein** kinase holoenzyme under equilibrium conditions: New insights into the **conformational changes** that result in cAMP-dependent activation. Johnson, David A.; Leathers, Valerie L.; Martinez, Anna M.; Walsh, Donal A.; Fletcher, William H. (Div. Biomed. Sci., Univ. California, Riverside, CA, 92521, USA). Biochemistry, 32(25), 6402-10 (English) **1993**. CODEN: BICHAW. ISSN: 0006-2960.

AB Previous studies of the ligand regulation of cAMP-dependent **protein** kinase (**protein** kinase A; PKA) demonstrated the cAMP-mediated dissocn. of the holoenzyme by using nonequil. techniques; i.e., gel filtration, ion-exchange chromatog., and differential centrifugation. While phys. mild, these could have caused weakly assocd. species to dissoc., thereby providing a potentially flawed interpretation of the mechanism of activation of PKA. To assess this, the activation of bovine heart PKA was monitored under equil. conditions using dipolar **fluorescence** energy **transfer** to measure **changes** in the proximity relations between the catalytic (C) and regulatory (R) subunits that compose the holoenzyme. Specifically, a heterochromatically **labeled** PKA type II holoenzyme was prepd. with the R and C subunits **labeled** with sulforhodamine and carboxyfluorescein, resp., and the exchange of electronic excitation energy between the C and R subunits was monitored by both donor lifetime and steady-state **fluorescence**. Biochem., the heterochromatic holoenzyme was closely identical to the native **protein** with regard to cAMP-induced increase in catalytic **activity**, reassocn. of C and R subunits, inhibition of catalytic **activity** by the specific **protein** kinase inhibitor (PKI) from rabbit muscle, and the obsd. dissocn. examd. by gel filtration upon cAMP addn. However, under equil. conditions, the energy-**transfer** measurements revealed that the addn. of cAMP to this heterochromatic reporter complex promoted an estd. 10-.ANG. increase in the distance between the derivatization sites on C and R but not a dissocn. of these subunits. The addn. of PKI plus cAMP promoted full dissocn. of the 2 subunits. The addn. of a high-affinity substrate [(Ser-21)PKI(14-22)-amide] had no significant effect on energy **transfer** and therefore the distance between derivatization sites on C and R. These results demonstrated (1) that, in vitro, cAMP does not decrease the binding affinity between the subunits of the holoenzyme as much as is generally assumed and (2) that PKI, but not a high-affinity substrate, can affect holoenzyme dissocn. To what extent these regulatory events occur in viable cells is currently being examd.

L109 ANSWER 21 OF 28 CAPLUS COPYRIGHT 2001 ACS
1991:601968 Document No. 115:201968 Flexibility of the aldolase molecule measured using quenching-induced variations of the Forster distance for

fluorescence energy **transfer**. Dobryszycki, Piotr; Krzyzanowska, Dorota; Kochman, Marian (Inst. Org. Phys. Chem., Tech. Univ., Wroclaw, 50-370, Pol.). Proc. - Indian Acad. Sci., Chem. Sci., 103(3), 435-40 (English) **1991**. CODEN: PIAADM. ISSN: 0253-4134.

AB The range of flexibility of the rabbit muscle aldolase mol. was studied using **fluorescent labeled** aldolase. The **protein** mol. was specifically **labeled** on the opposite sites of the **enzyme** subunit with **fluorescence** energy donor and acceptor residues. **Labeled** aldolase with full enzymic **activity** was used as a tool in the **FRET** studies between 1,5-IAEDANS (donor) on Cys-289 and 5-iodoacetamidofluorescein (acceptor) on Cys-239. A range of Forster distances (R) were obtained by collisional quenching of the donor emission. The expts. of donor **fluorescence** quenching with a wide range of acrylamide concns. showed the variations in donor-acceptor (D-A) distances. In the absence of quencher, the D-A distance distribution was characterized by an av. value of 40.4 .ANG., and a half-width of 0.13 .ANG.. A dramatic increase in half-width to 17.7 .ANG. was obsd. after exposure of the **enzyme** to high acrylamide concns. (0.13-0.68M).

L109 ANSWER 22 OF 28 CAPLUS COPYRIGHT 2001 ACS

1991:578841 Document No. 115:178841 Immobilization of molecular binding partners to contact activating supports. Motsenbocker, Marvin A. (USA). U.S. US 5043288 A **19910827**, 7 pp. (English). CODEN: USXXAM. APPLICATION: US 1988-208984 19880620.

AB Mol. binding partners (e.g. antibodies, antigens, avidin, biotin) are immobilized to support materials that have the property of contact activation of blood protein coagulation. A binding partner is attached to a surface-active protein carrier (e.g. factor XII, fibrinogen, fibronectin), and the resulting conjugate is noncovalently adsorbed onto the surface of the support. The method provides for diagnostic assays of greater sensitivity and convenience. Thus, a conjugate of factor XII with antibody against human chorionic gonadotropin .alpha. chain was prepd. and used to coat the inner surface of glass test tubes. The conjugate-coated tubes performed better in immunoassays than tubes coated with antibody alone.

L109 ANSWER 23 OF 28 CAPLUS COPYRIGHT 2001 ACS

1990:51823 Document No. 112:51823 Preparation of ligand-polymer conjugate having a controlled number of introduced ligands, and its use in immunoassays. Lewis, Lynette A.; Yip, Kin Fai (Miles, Inc., USA). Eur. Pat. Appl. EP 317796 A1 **19890531**, 16 pp. DESIGNATED STATES: R: DE, FR, GB, IT. (English). CODEN: EPXXDW. APPLICATION: EP 1988-118110 19881031. PRIORITY: US 1987-118566 19871109.

AB A method is provided for chem. coupling a controllable no. of ligands to a polymeric material. The method comprises (1) obtaining a polymeric material having repeating functional groups; (2) derivatizing the functional groups to introduce a controllable proportion of a 2nd functional group; and (3) coupling the ligands to the polymeric material

through the introduced 2nd functional groups. The 2nd functional group is added by reacting the polymeric material with a mixt. of a predetd. ratio of excess amts. of (1) an activating agent which is the 2nd functional group, and (2) a blocking agent. A particle agglutination inhibition immunoassay, e.g. for Hb A1c, using an agglutinator reagent prepd. as above is also described. Ability to attach a controllable high d. of ligand on the polymer results in immunoassays of improved sensitivity and precision. Thus, aminoethanol and 4,9-dioxa-1,12-dodecanediamine in DMF were treated with poly(aspartic acid) in the same solvent. Following reaction at room temp. for 1 h and at 70.degree. for 2 h, the crude product was isolated and purified with P6-DG desalting gel chromatog. Three prepns. gave 11.7, 22, and 36.7 amino groups/mg polymer. The amino-functionalized poly(aspartic acid) was reacted with 4-(maleimidomethyl)-1-cyclohexanecarboxylic acid-N-hydroxysuccinimide ester; the activated polymer was sepd. on a P6-DG column, then reacted with a glycated hexapeptide (prepd. in European Patent Publication 185,870). The product was purified on the above chromatog. column and used in a latex agglutination assay for Hb A1c detn. Reproducible max. agglutinations were dependent on the controlled no. of ligand residues in the polymer.

L109 ANSWER 24 OF 28 CAPLUS COPYRIGHT 2001 ACS

1987:632131 Document No. 107:232131 2'-Deoxy-3'-O-(4-benzoylbenzoyl)- and 3'(2')-O-(4-benzoylbenzoyl)-1,N6-ethenoadenosine 5'-diphosphate, **fluorescent** photoaffinity analogs of adenosine 5'-diphosphate. Synthesis, characterization, and **interaction** with myosin subfragment 1. Cremo, Christine R.; Yount, Ralph G. (Inst. Biol. Chem., Washington State Univ., Pullman, WA, 99164-4660, USA). Biochemistry, 26(23), 7524-34 (English) **1987**. CODEN: BICHAW. ISSN: 0006-2960.

AB Two new **fluorescent** nucleotide photoaffinity **labels**, 3'(2')-O-(4-benzoylbenzoyl)-1,N6-ethenoadenosine 5'-diphosphate (Bz2.epsilon.ADP) and 2'-deoxy-3'-O-(4-benzoylbenzoyl)-1,N6-ethenoadenosine 5'-diphosphate [3'(Bz2)2'd.epsilon.ADP], were synthesized and used as probes of the ATP-binding site of myosin subfragment 1 (S1). These analogs were stably trapped by the bifunctional SH group crosslinker, N,N'-p-phenylenedimaleimide (pPDM) at the active site in a manner similar to that of previously shown for ATP, and nonspecific photolabeling could be minimized by removing the free probe by gel filtration prior to irradn. Both probes covalently photoincorporated with high efficiency (40-50%) into the central 50-kilodalton heavy chain tryptic **peptide**, as found previously for the nonfluorescent parent compd., 3'(2')-O-(4-benzoylbenzoyl)ADP. The soln. **conformations** of Bz2.epsilon.ADP and 3'(Bz2)2'd.epsilon.ADP were analyzed by steady-state and time-resolved **fluorescence** spectroscopy. The benzoylbenzoyl rings in both analogs were stacked over the .epsilon.-adenine ring. The degree of stacking was greater with the 2' isomer than with the 3' isomer. **Fluorescence** quantum yields and lifetimes were measured for Bz2.epsilon.ADP and 3'(Bz2)2'd.epsilon.ADP reversibly bound, stably trapped, and covalently photoincorporated at the active site of S1. These values were compared with those for 3'(2')-O-[[(phenylhydroxymethyl)phenyl]carbonyl]-1,N6-ethenoadenosine diphosphate (CBH.epsilon.ADP) and 2'-deoxy-3'-O-

[[(phenylhydroxymethyl)phenyl]carbonyl]-1,N6-ethenoadenosine diphosphate [3'(CBH)2'd.epsilon.ADP]. These derivs. were synthesized as **fluorescent** analogs of the expected product of the photochem. **reactions** of Bz2.epsilon.ADP and 3'(Bz2)2'd.epsilon.ADP, resp., with the active site of S1. The **fluorescence** properties of the carboxybenzhydrol derivs. trapped at the active site by pPDM were compared with those of the Bz2 nucleotide-S1 complexes. These properties were consistent with a photoincorporation mechanism in which the carbonyl of benzophenone was converted to a tertiary alc. attached covalently to the **protein**. The specific, highly efficient photoincorporation of Bz2.epsilon.ADP at the active site will allow it to be used as a donor in distance measurements by **fluorescence resonance energy transfer** to acceptor sites on actin.

L109 ANSWER 25 OF 28 CAPLUS COPYRIGHT 2001 ACS
1986:621693 Document No. 105:221693 **Fluorescence** studies of chicken liver fatty acid synthase. Segmental flexibility and distance measurements. Yuan, Zhengyu; Hammes, Gordon G. (Dep. Chem., Cornell Univ., Ithaca, NY, 14853, USA). J. Biol. Chem., 261(29), 13643-51 (English) **1986**. CODEN: JBCHA3. ISSN: 0021-9258.

AB The 4'-phosphopantetheine of chicken liver fatty acid synthase was specifically **labeled** with the **fluorescent** substrate analog, CoA 6-[7-nitrobenz-2-oxa-1,3-diazol-4-yl]aminohexanoate at low salt concns. A serine at the active site of the thioesterase was specifically **labeled** with the **fluorescent** compds. 6-[7-nitrobenz-2-oxa-1,3-diazol-4-yl]aminopentylmethylphosphonofluoridate and(or) pyrenebutyl methylphosphonofluoridate. Dynamic anisotropy measurements indicated that the thioesterase had considerable segmental flexibility, whereas the **fluorescent-labeled** 5'-phosphopantetheine did not display detectable local or segmental flexibility. **Fluorescence resonance energy transfer** measurements indicated that the distance between the **fluorescent label** at the end of the 4'-phosphopantetheine and NADPH bound to the .beta.-ketoacyl reductase or enoyl reductase site on the same **polypeptide** chain was essentially the same, .apprx.35 .ANG.. The 2 types of reductases were distinguished by specifically blocking enoyl reductase with pyridoxal 5'-phosphate. No significant energy **transfer** occurred between sites on different **polypeptide** chains so that the distances must be >55 .ANG.. The distance between the serine on the thioesterase and the 4'-phosphopantetheine on the same **polypeptide** was 48 .ANG.; again no interpolypeptide chain energy **transfer** was obsd. The distance between the serines of the 2 thioesterases within a fatty acid synthase mol. was >56 .ANG.. The monomeric **enzyme** obtained at 1.degree. did not have .beta.-ketoacyl synthase and reductase **activities**. Also **fluorescent** titrns. indicated that NADPH was not bound to .beta.-ketoacyl reductase in monomeric **enzyme**. The addn. of K phosphate to the monomers at 1.degree. rapidly dimerized the **enzyme** and restored the .beta.-ketoacyl reductase **activity**. The .beta.-ketoacyl synthase **activity** was slowly restored when the dimer was warmed to room temp. The results obtained suggested that relatively large **conformational changes** may be part of the catalytic cycle.

L109 ANSWER 26 OF 28 CAPLUS COPYRIGHT 2001 ACS
1982:419803 Document No. 97:19803 The active site structure of sodium ion- and potassium ion-stimulated ATPase. Location of a specific fluorescein isothiocyanate reactive site. Carilli, Cynthia T.; Farley, Robert A.; Perlman, David M.; Cantley, Lewis C. (Dep. Biochem. Mol. Biol., Harvard Univ., Cambridge, MA, 02138, USA). J. Biol. Chem., 257(10), 5601-6 (English) **1982**. CODEN: JBCHA3. ISSN: 0021-9258.

AB Fluorescein 5'-isothiocyanate (I) has previously been shown to specifically inactivate (Na+,K+)-ATPase at low concns. The site of modification of purified dog kidney (Na+,K+)-ATPase by I was investigated by enzymic cleavage and **fluorescence resonance energy transfer**. The binding of I, which occurred at a stoichiometry of .apprx.1 site/ATP binding site, caused an ATP-protectable inactivation of ATPase **activity**, suggesting that it was reacting at the ATP hydrolysis site. The I **reaction** site apparently was located near the center of the C-terminal 77,000-dalton **peptide** fragment obtained by chymotryptic cleavage of the .alpha. subunit. Addn. of ouabain to the native **enzyme** in the presence of chymotrypsin enhanced cleavage at this site and released the fluorescein moiety from the membrane. It was further shown that the distance from the I **reaction** site to the ouabain binding site, as judged by **fluorescence resonance energy transfer** from anthroylouabain to I, was .apprx.74 .ANG.. Thus, ouabain inhibits (Na+,K+)-ATPase by causing a **protein conformational change** which extends an unusually large distance across the membrane.

L109 ANSWER 27 OF 28 CAPLUS COPYRIGHT 2001 ACS
1981:510954 Document No. 95:110954 Application of photoactivatable **fluorescent** active-site directed probes to serine-containing **enzymes**. Angelides, Kimon J. (Dep. Biochem., McGill Univ., Montreal, PQ, H3G 1Y6, Can.). Biochim. Biophys. Acta, 669(2), 149-56 (English) **1981**. CODEN: BBACAQ. ISSN: 0006-3002.

AB A photoactivatable **fluorescent** anthraniloyl group has been directed to the active site serine group of .alpha.-chymotrypsin and trypsin. The acylated derivs. are nonfluorescent until irradiated. When activated by light a highly reactive nitrene is generated which is capable of covalent insertion into the **protein** matrix. The resultant insertion product of this photolysis is a highly **fluorescent** reporter group which has little rotational mobility and is crosslinked through the serine to the **protein** matrix in the active site region of the **protein**. Because of the sensitivity to the polarity of the environment shown by the anthraniloyl chromophore, the dipolar relaxation characteristics of the crosslinked **enzyme** and deacylated **enzyme** were detd. These measurements show that little relaxation occurs on the nanosecond ime scale for the crosslinked **enzyme**, but on deacylation of the serine increased dipolar relaxation of the **protein** with the attached reporter group is obsd. The use of these active-site directed photoactivatable **fluorescent** probes can be extended to probe the active-site structure of complex **enzymes** and **conformational** dynamics of active-site regions in **proteins** and to serve as potential functional site **labels** in **fluorescence**

resonance energy transfer measurements.

L109 ANSWER 28 OF 28 CAPLUS COPYRIGHT 2001 ACS
1980:527920 Document No. 93:127920 Structural **changes** in (sodium-potassium ion) dependent ATPase accompanying detergent inactivation. Powell, Leland D.; Cantley, Lewis C. (Dep. Biochem. Mol. Biol., Harvard Univ., Cambridge, MA, 02138, USA). Biochim. Biophys. Acta, 599(2), 436-47 (English) **1980**. CODEN: BBACAQ. ISSN: 0006-3002.
AB Structural **changes** in Na+ + K+-ATPase purified from eel elec. organ microsomes accompanying detergent inactivation were investigated by monitoring **changes** in light scattering, intrinsic **protein fluorescence**, and tryptophan-to-.beta.-parinaric acid **fluorescence resonance energy transfer**. Two phases of inactivation were obsd. using the nonionic detergents, digitonin, Lubrol WX, and Triton X-100. The rapid phase involves detergent monomer insertion but little **change** in **protein** structure or little displacement of closely assocd. lipids as judged by intrinsic **protein fluorescence** and **fluorescence resonance energy transfer**. Lubrol WX and Triton X-100 also caused membrane fragmentation during the rapid phase. The slower phase of inactivation results in a completely inactive **enzyme** in a particle of 400,000 daltons with 20 mol/mol of assocd. phospholipid. **Fluorescence changes** during the course of the slow phase indicate some dissocn. of **protein**-assocd. lipids and an accompanying **protein conformational change**. Nonparallel inhibition of (Na+ + K+)-ATPase and p-nitrophenylphosphate **activity** by digitonin (which occurs during the rapid phase of inactivation) is unlikely to require a **change** in the oligomeric state of the **enzyme**. At least 20 mol/mol of tightly assocd. lipid mols. are necessary for either (Na+ + K+)-ATPase or p-nitrophenylphosphatase **activity** and the rate-limiting step in the slow inactivation phase involves dissocn. of an essential lipid.

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